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Arbuscular mycorrhizal fungi from northeast India

Panna, D. and Highland, K.*

Microbial Ecology Laboratory, Botany Department, North Eastern Hill University, Shillong-793 022, India.

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Arbuscular mycorrhizal fungi (AMF) established ubiquitous symbiotic associations with the majority of plants. Nevertheless, AMF are less known from northeast India. In this communication, sporocarpic species of AMF were extracted and described from the rhizosphere of *Alnus nepalensis*, *Michelia champaca* and from the cultivation field of *Solanum tuberosum*. The spores were isolated using wet sieving and decanting method. Ten sporocarpic species were isolated and described. Nine morphotypes belong to the genus *Glomus* and only one unidentified. The identified morphotypes were *Glomus aureum, G. clavisporum, G. fuegianum, G. glomerulatum, G. macrocarpum, G. microaggregatum, G. rubiforme, G. sinuosum and G. taiwanense.*

Key words: Arbuscular mycorrhizal fungi, northeast India, sporocarpic species, *Glomus*, morphotypes

Introduction

Arbuscular mycorrhizal fungi (AMF) are members of the fungal phylum Glomeromycota (Schüßler *et al.*, 2001). AMF are ubiquitous in terrestrial ecosystems, forming symbiotic associations with roots from the majority of plants (Smith and Read, 1997). These associations are mainly interested because of the manifold benefits conferred on the host by the fungus. They are known to improve the nutritional status of plants and growth and development, protect plants against root pathogens and confer resistance to drought and soil salinity conditions (Bagyaraj and Varma, 1995).

Most of the species from sporocarpic genus *Sclerocystis* was transferred to the genus *Glomus* (Almeida and Schenck, 1990). Many AMF, often sporocarpic species, are not well known regarding their distribution (Goto and Maia, 2005). Nevertheless, studies on AMF are meager from northeast India. In

*Corresponding author: Highland K; e-mail address: hkayang@nehu.ac.in

this present communication, sporocarpic species of AMF were surveyed from several soils on this region.

Materials and methods

The soil samples were collected from six sites in Meghalaya, India during 2007 (Fig. 1). The soils were analysed for extraction of sporocarpic species of AMF. Three soils replicates from each of the six sites were sampled to examine the sporocarpic species of AMF. The samplings were done from four plantation sites of *Michelia champaca* Linnaeus, one each from *Alnus nepalensis* Don and *Solanum tuberosum* Linnaeus. The soil sample from each site was made into one composite soil sample and transported to laboratory for analysis. Spore extraction was done from the 100g of soil samples from the six sites following wet sieving and decanting method (Gerdemann and Nicolson, 1963).

The isolated spores were picked up with needle under a dissecting microscope and were mounted in polyvinyl alcohol–lactoglycerol (Koske and Tessier, 1983) and also in mixed polyvinyl alcohol–lactoglycerol and Meltzer's reagent (1:1, v:v) for identification. The complete and broken spores were examined using a Olympus compound microscope. All the spores were photographed with the help of Leica EC 3 camera attached in Leica dm 1000 microscope. Taxonomic identification of spores to species level was based on sporocarpic size, colour, ornamentation and wall characteristics by matching original descriptions (Koske, Gemma *et al*., 1986; Almeida and Schenck, 1990; Wu *et al*., 1995; Oehl and Sieverding 2004). All the permanent slides were deposited in the Microbial Ecology Laboratory, Botany Department, North Eastern Hill University, Shillong.

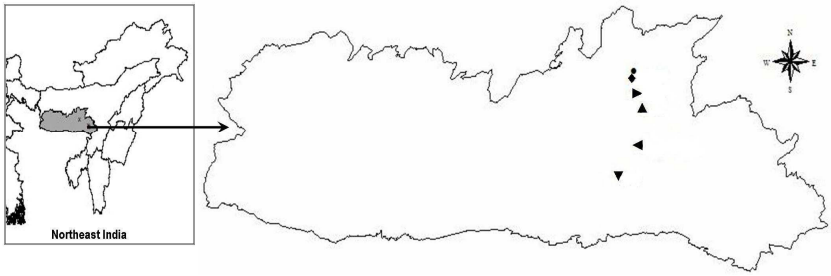

Fig. 1. Map of northeast India showing study sites in Meghalaya Whereas; ● -Umdihar; Umsaw; ► -Mawlein; ▲ -ICAR; ◄ -Upper Shillong; ▼-Swer.

Results

Ten sporocarpic species were extracted from soil and taxonomically described. Nine morphotypes belong to the genus *Glomus* and only one unidentified; *Glomus aureum, G. fuegianum, G. clavisporum, G. glomerulatum, G. macrocarpum, G. microaggregatum, G. rubiforme, G. sinuosum, and G. taiwanense.* Seven species were isolated from the rhizosphere of *Michelia champaca,* five species from the agricultural field of *Solanum tuberosum* and two species from the rhizosphere of *Alnus nepalensis*. *Glomus rubiforme* was found from five sites. *Glomus clavisporum* and *G. macrocarpum* were recovered from three sites. *Glomus microaggregatum and G. glomeratum* were recovered from two sites; *Glomus aureum, G. fuegianum, G. sinuosum, G. taiwanense* and unidentified species from only one site. The species were described as follows:

Glomus aureum Oehl, Wiemken & Sieverding, J. Appl. Bot. 77: 111-115, 2003

Sporocarp size 300µm, light orange; irregular in shape; without a peridium; comprises of quite closely packed spores. Within the sporocarps consists of interwoven hyphae, which are hyaline to yellow straw. Spores and hyphae are embedded in an amorphous gelly material staining pastel red in Melzer's reagent. Spores formed blastically at the tip of either dichotomously branched hyphae usually ovoid 45-50 µm with one subtending hypha (Fig. 2a). Spores are of two-layered wall (Fig. 2b). Layer 1, forming the spore surface, evanescent, hyaline usually slightly sloughed in spores. Layer 2 light orange, 2-4 µm thick, up to 5.6 µm thick at the spore base.

Distribution: Extracted from the rhizosphere soil of *Alnus nepalensis* grown as landscape trees along the road located in Upper Shillong.

Glomus clavisporum (Trappe) Almeida & Schenck**,** Mycologia 82:710, 1990

Sporocarps globose 370 x 360 µm brownish black (Fig. 2c); tightly pack around a central plexus of interwoven hyphae. Peridium absent. Chlamydospores brown to dark brown 90-160x35-45 µm, clavate to subcylinderic tapering to cylinderic subtending hyphae. Spore wall laminate, 2.5-6.0 µm thick on the side walls, thickened 16-22 µm at the apex and 5-9 µm at the base. Two distinctly different sized spores (Fig. 2d). Central plexus 120-150 µm diameter. No distinct reaction with Meltzer's reagent.

Distribution: Found from the soils of agroforestry system in the rhizosphere of *Michelia champaca* located in Indian Council of Agricultural Research (ICAR) in Umiam, from soils of plantation of *Michelia champaca* situated in Umdihar and also from Swer, potato field.

Glomus fuegianum (Spegazzini) Trappe & Gerdemann, Mycol. Mem. 5:58, 1974

Sporocarp containing 7 spores radially arranged and tightly adherent spores developed from a thick-walled, inflated hypha. Sporocarp light brown; 430 x 440µm; with a peridium (Fig. 2e). Peridium hyaline**;** interwoven hyphae, usually present only on a part of a sporocarp. Spores yellowish brown; ovoid 80-150 µm; with a single subtending hypha; spores frequently surrounded by branched and convoluted hyphae. The wall composed of two layers (Fig. 2f). Layer 1 evanescent, smooth, hyaline, 0.9- 1.0 thick. Layer 2 laminate, yellowish brown, 3-5 µm thick. Two layers do not stain in Melzer's reagent.

Distribution: Isolated from potato field situated in Swer village.

Glomus glomerulatum Sieverding, Mycotaxon 29:73-79, 1987

Sporocarps dark brown and irregular formed by interwoven, straight, curved and branched, pale orange hyphae, 550 µm thick (Fig. 2g). Sporocarps without a peridium. The interior of the sporocarps filled with an amorphous, colourless substance and with soil debris. Spores brown; globose to subglobose; 50-65 µm diam. Two spore wall present. Layer 1 light orange to golden yellow, 5-10 µm thick. Layer 2 flexible, hyaline, usually tightly adherent to the inner surface of layer 1. Layers 1 and 2 do not stain in Melzer's reagent. The distinctive morphological character of *G. glomerulatum* is the formation of small and coloured spores only intercalary along its sporocarpic hyphae. Hence, all spores of this species always have two subtending hyphae (Fig. 2h).

Distribution: Found from the plantation of *Michelia champaca* in Mawlein and also from soils of plantation of *Michelia champaca* situated in Umdihar.

Glomus macrocarpum Tulasne & Tulasne**,** Giorn. Bot. Ital. 2:55-63, 1844

Sporocarps in 4-15 distributed spores 350 µm in crushed state (Fig. 2i). Spores yellow subglobose; 110-115 diam; mostly with one subtending hypha. Peridium not found. Additionally, the spore wall structure of *G. macrocarpum* comprises two layers, of which outer layer is hyaline (Fig. 2j). Layer 1 semi flexible, hyaline, 1.83 µm thick. Layer 2 smooth, yellow, 4.29 µm thick. Layers 1 and 2 do not react in Melzer's reagent.

Distribution: Found from the rhizosphere soils of *Michelia champaca* in Umdihar, from soils of plantation of same species situated in Umsaw under state Forestry Department and also from potato field situated in Swer.

Glomus microaggregatum Koske, Gemma & Olexia, Mycotaxon 26:125-132, 1986

Sporocarp identified by its small size, hyaline colour of the spore; crushed sporocarp 600 µm (Fig. 2k). Spore diameter 40-40 µm. Wall thickness 1.3-2µm.

Distribution: Isolated from potato field situated in Swer village and from the soils of plantation of *Michelia champaca* in Umsaw.

Glomus rubiforme (Gerdemann & Trappe) Almeida & Schenck, Mycologia 82:709-710, 1990

Spores occur in sporocarp in the soil. Sporocarps yellow brown; subglobose; 190-250 µm diam without a peridium; with 22-24 spores. Spores light brown; globose to subglobose; 44-57 µm diam; with a single subtending hypha; developed from a thick-walled, inflated hypha, wall thickness 5-10 µm; spores arranged radially to form a blackberry-like sporocarp when mature (Fig. 2l).

Distribution: Found in five sites such as the plantation of *Michelia champaca* in Umdihar, Umsaw and Mawlein; in rhizosphere soil of *Alnus nepalensis* in Upper Shillong and in Swer from potato field.

Glomus sinuosum (Gerdemann & Bakshi) Almeida & Schenck, Mycologia 82:710-711, 1990.

Sporocarp brown and irregular surface due to protruding spores covered by a dense peridium enclosing tightly, composed of thick walled, septate sinuous hyphae. 600 µm in crushed condition (Fig. 2m). Chlamydospores usually were clavate to elliptical radiating side by side in a single layer of 45-85 µm. Chlamydospores are pale yellow, wall laminate 11-13 µm in diameter generally thickened at the base. Reaction to Meltzer's reagent not distinct.

Distribution: Found from the plantation of *Michelia champaca* situated in Umdihar.

Glomus taiwanense (Wu & Chen) Almeida & Schenck, Mycologia 82:711-712, 1990.

Sporocarp was recovered from the soil with only four chlamydospores 200 µm. Chlamydospores formed radially in a single, tightly packed layer around a central plexus of hyphae 150 µm. Chlamydospore outer wall is reddish brown and inner is light brown. Chlamydospores of 110x50 µm wall with a hyaline, separable outer layer (Fig. 2n). Wall at the apex 17-20 and side 5-11.

Distribution: From plantation of *Michelia champaca* situated in Umdihar.

Unidentified species

Sporocarp is irregular. Spore clusters 50-90 µm (Fig. 2o). Spores are shiny bright in colour; 65-120 µm. Outer layers have very sharp spines on it (Fig. 2p). Spine size 18-20 µm with 11-15 µm at the base and 1.8-2.5 µm at the tip.

Distribution: From plantation of *Michelia champaca* situated in Mawlein.

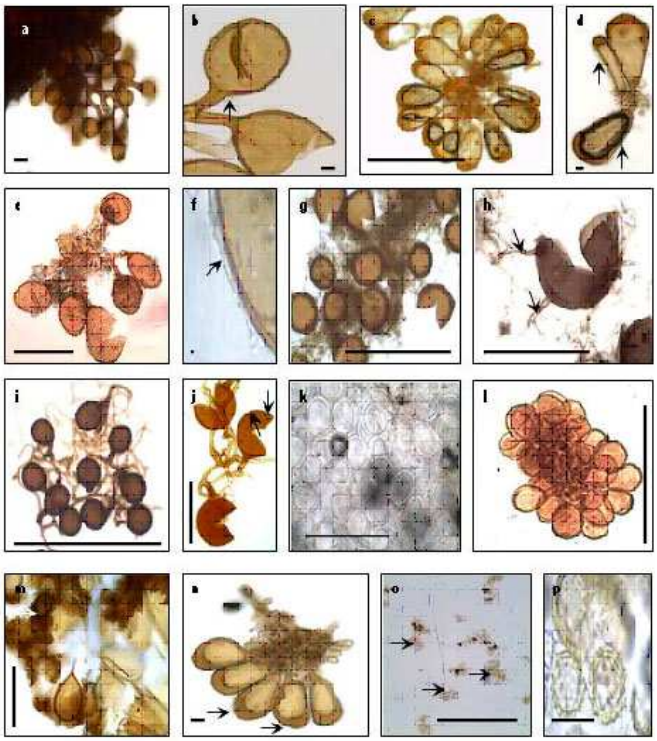

Fig. 2. (a-p) AMF species isolated from different sites.(a) Portion of sporocarp of *Glomus aureum*. Bar=50 µm; (b) Disintegrating hyaline outer wall of *G. aureum* Bar=50 µm; (c) Portion of sporocarp of *G. clavisporum* Bar=270 µm; (d) Different spore types of *G. clavisporum* Bar=30 µm (e) Sporocarp of *G. fuegianum* Bar=200 µm (f) Outer hyaline layer of *G. fuegianum* Bar=10 µm. (g) Portion of sporocarp of *Glomus glomeratum* Bar= 350 µm; (h) Two hyphae of *G. glomeratum* Bar= 350 µm; (i) Sporocarp of *G. macrocarpum* Bar= 500 µm; (j) Spores of *G. macrocarpum* showing two wall layers. Bar= 250 µm; (k) Spores of *G. microaggregatum* Bar=300 µm; (l) Black berry like structures of sporocarps of *G. rubiforme*. Bar=530 µm; (m) Spores of *G. sinuosum* showing sinuosum hyphae and clavate spore. Bar=300 µm; (n) Five spores of *Glomus taiwanense* showing hyaline wall layer. Bar=50 µm; (o & p) Spores of unidentified species. Bar=300 and 150 µm, respectively.

Discussion

The hyaline layer of *Gomus aureum* is found in sloughed condition as described (Oehl *et al.*, 2003). Two distinctly different sized spores are formed in *G. clavisporum* and hyaline separable layer in *G. taiwanense* differentiate two species as described (Wu, 1993). Only one sporocarp of *G. fuegianum* was isolated in this study which is also found to be an extremely rarely occurring in the world. The spores of *Glomus glomerulatum* always have two subtending hyphae is in accordance with the report (Sieverding, 1987). *G. macrocarpum* are similar in size and colour; moreover, the spore wall structure comprises of two layers. Blackberry-like sporocarp structure in *G. rubiforme* confirmed with the report (Almeida and Schenck, 1990). Peridium tightly enclosing a sporocarp composed of thick-walled interwoven sinuous hyphae as described (Almeida and Schenck, 1990). Bright colour and ornamentation is the characteristic feature of *Pacispora* (Oehl and Sieverding, 2004) which suggest that the unidentified species may belong to *Pacispora*. However, sporocarpic nature is not known in the genus. Trap culture of the rhizosphere soil from where this species is isolated would give apparent observation to taxonomically describe the species more elaborately to assign a specific taxon to it.

The study of AMF in northeast India is meager apart from studies of Sharma *et al*. (1984 and 1986) who also assumed that these soils are dominated by *Glomus*. Most of the species were isolated from the rhizosphere soils of *Michelia champaca*, being the member of Magnoliales which has coarsely branched root structure response to mycorrhizal infection efficiently (St John, 1980) may also facilitate more diverse AMF community. Therefore, a comprehensive study of AMF associated with *Michelia champaca* is required further study.

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